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Contamination of Red-Meat Carcasses by *Campylobacter fetus* subsp. *jejuni*

C. O. GILL* AND LYNDA M. HARRIS

Meat Industry Research Institute of New Zealand, Inc., Hamilton, New Zealand

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Campylobacter fetus subsp. *jejuni* was commonly present in the feces of unweaned calves (2 to 3 weeks old) and from two of four groups of sheep. One new season lamb (12 to 16 weeks old) carried the organism, but the bacteria were not isolated from cattle. With unweaned calves, the fractions of animals infected and carcasses contaminated were similar. Contamination of carcasses usually involved low densities of *C. fetus* subsp. *jejuni* (ca. 1 to 10/cm^2), which were isolated from flank but not rump areas. The organism was recovered less frequently from chilled carcasses and deboned veal. Small numbers of *C. fetus* subsp. *jejuni* could be recovered from equipment during the processing of unweaned calves but not after routine cleaning.

The development of simple techniques for the isolation of *Campylobacter* species from feces has resulted in the recognition of *Campylobacter* enteritis as a common enteric infection of humans (8). The causative organisms are distinguished from other groups of *Campylobacter* by their ability to grow at 43°C (9). These organisms have been variously classified as *C. fetus* subsp. *jejuni* (10) and as two distinct species, *C. jejuni* and *C. coli* (15). A second *C.* group, *C. fetus* subsp. *intestinalis* (10) or *C. fetus* subsp. *fetus* (15), can cause systemic disease in humans, but infection by these organisms is apparently restricted to patients with compromised immune-response mechanisms (1).

Both of these organisms can cause disease in animals, but *C. fetus* subsp. *jejuni* is the organism of major concern for human health. It is believed to be transmitted orally, but the epidemiology of *Campylobacter* enteritis is still obscure. The organism can be carried in the intestines of apparently healthy animals and birds (11), so animal-associated foods are likely to be implicated in its transmission. There is strong evidence linking specific outbreaks of *Campylobacter* enteritis to the consumption of unpasteurized milk (5), and since much chicken meat can be contaminated with strains of *Campylobacter* (3) and the organism is common in healthy pigs (4) both poultry and pork have been suggested as vectors. However, only limited data have been published on the contamination of red-meat carcasses by *C. fetus* subsp. *jejuni* (12, 13). Since data on the extent and degree of contamination of red-meat carcasses by this organism are necessary for the assessment of any health risk to humans, we examined such carcasses and the environment at a commercial abattoir for the presence of *Campylobacter*.

MATERIALS AND METHODS

Media. *Campylobacter* strains were isolated on plates of Albimi Brucella agar (Pfizer Inc., New York) containing 5% human whole blood and a *Campylobacter* growth supplement of sodium pyruvate, sodium metabisulfite and ferrous sulfate, all at 250 mg/liter (Oxoid Ltd, Basingstoke, England). This *Campylobacter* agar was rendered selective by the addition of antimicrobial agents: either those recommended by Skirrow (vancomycin, polymyxin, and trimethoprim [Oxoid *Campylobacter* selective supplement SR69]) or those recommended by Butzler (bacitracin, cyclohexamide, colistin sulfate, cephazolin sodium and, novobiocin [Oxoid *Campylobacter* selective supplement SR85]).

Examination of feces. Fecal samples were obtained from viscera shortly after they were removed from carcasses. Samples of feces (5 g) were macerated with 45 ml of 0.1% peptone with a Colworth stomacher and serial dilutions to 10^{-3} prepared in peptone water. Samples (0.1 ml) of the original macerate and each dilution were spread on four plates of each selective *Campylobacter* agar. Two plates of each set were incubated at 37°C, and two were incubated at 43°C for up to 4 days, each in an atmosphere of 5% O_2, 10% CO_2, and 85% N_2. The identities of suspect *Campylobacter* colonies were confirmed by dark-background microscopic examination of wet slide preparations (1). All isolates were inoculated onto six plates of *Campylobacter* agar without antimicrobial supplements. Two plates of each set were incubated under the reduced oxygen atmosphere at 25, 37 or 43°C.

Examination of meat and work surface. Sterile swabs moistened with 0.1% peptone were used to sample areas of 100 cm^2 of meat and work surfaces. Dry swabs were used where work surfaces were wet. Each swab was spread directly onto the surface of a plate of

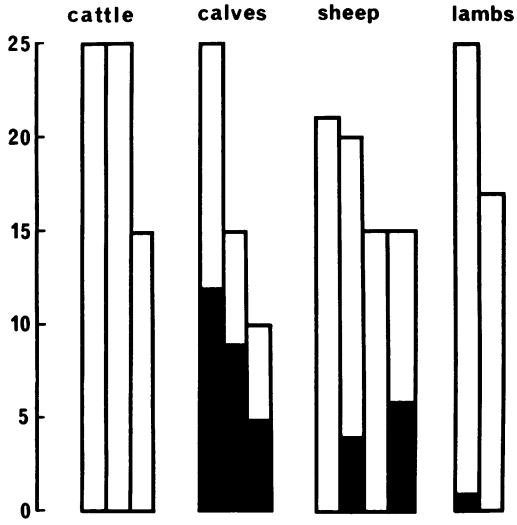

FIG. 1. Occurrence of *C. fetus* subsp. *jejuni* in feces from slaughtered animals. Symbols: □ number of animals sampled; ■, number positive for *C. fetus* subsp. *jejuni*.

Campylobacter agar containing the antimicrobial supplement of Skirrow. The plates were incubated under the reduced-oxygen atmosphere for 2 days at 37°C. *Campylobacter* colonies were identified and subcultured as for fecal samples.

The efficacy of the sampling technique was tested by recovering *C. fetus* subsp. *jejuni* from glass plates and beef surfaces over which inocula (0.1 ml) of known cell densities had been spread.

RESULTS

Feces from cattle, unweaned calves, (2 to 3 weeks old) sheep, and new season lambs (12 to 16 weeks old) were examined. Samples were collected from at least two distinct groups of each type of animal. *Campylobacter* species were present in all groups of calves. Two of four groups of sheep included several animals carrying the organism, but only one positive sample was obtained from 42 lambs and none were obtained from 65 cattle (Fig. 1). In all cases the organisms grew at 43°C but not at 25°C, showing that they were strains of the *C. fetus* subsp. *jejuni* group. Identical results were obtained with media containing either the Butzler or Skirrow antibiotic supplements, but visible colonies developed within 48 h with the latter supplement, whereas 3 to 4 days were required with the former. The antibiotic supplement of Skirrow was therefore used for subsequent isolations.

Since the *Campylobacter* strains were consistently present only in unweaned calves, carcasses from these animals were used to study the spread and survival of *C. fetus* subsp. *jejuni* within the abattoir. Swab samples were taken from different groups of 10 carcasses at four points during processing on 3 days. Fecal samples were also obtained from 10 animals on each day. Positive fecal and carcass samples were obtained each day. Carcasses were swabbed at both the flank (abdominal wall) and rump regions, but all samples from the rump gave negative results. Overall, a similar number of positive fecal and carcass samples were obtained before the carcasses were chilled. Only half as many positive samples were obtained from carcasses subjected to overnight chilling. Cell densities in feces were 10^3/g or less. Generally, only unit numbers of colonies were recovered from positive carcass sites (Table 1).

Examination of the environment showed that small numbers of *C. fetus* subsp. *jejuni* could be isolated on some occasions from a variety of sites. However, the only surfaces with a high density and rate of contamination were full viscera trays. The rate and degree of contamination of trays decreased markedly with routine washing (Table 2). No positive results were obtained on two occasions when surfaces were sampled after cleaning up after calf kills.

The swabbing technique had a recovery rate of about 1% for meat surfaces, although recov-

TABLE 1. Contamination by *C. fetus* subsp. *jejuni* of unweaned calf carcasses and boneless veal[a]

Point of sampling	Mode of sampling	No. positive from 30 samples	Individual counts of *C. fetus* subsp. *jejuni*[b]	Average no. of bacteria[b]
Feces	Maceration	6	5×10^2, 30×10^2, 50×10^2, 1×10^2, 2×10^2, 20×10^2	1.6×10^3
After skinning	Swabbing	7	90, 10, 3, 2, 2, 2, 1	15.7
After washing	Swabbing	6	22, 5, 4, 2, 1, 1	5.8
After chilling	Swabbing	3	2, 1, 1	1.3
After deboning	Swabbing	4	25, 4, 2, 1	8

[a] Samples from meat were obtained by swabbing 100 cm^2 of the flank region of carcasses. Samples were taken from separate groups of 10 animals at each stage of processing on 3 days.

[b] Bacterial counts were measured as number of bacteria per 100 cm^2 of sample, except for feces samples, which were measured as number of bacteria per gram of feces.

TABLE 2. Contamination of the environment by *C. fetus* subsp. *jejuni* during processing of unweaned calf carcasses

Sampling site	No. of samples	No. positive	Individual counts (No./100 cm^2)
Waste chute	4	0	
Carcass drainage area	3	2	5, 5
Viscera trays (clean)	7	1	11
Viscera trays (full)	9	5	200×10^2, 18×10^2, 4×10^2, 3×10^2, 0.1×10^2
Deboning tables	12	2	4, 1
Boning room conveyors	12	3	2, 1, 1

[a] Samples were taken by swabbing 100 cm^2 of each site on 3 days.

ery of single colonies could occur with total numbers substantially below 100. The recovery rate from glass surfaces was about 10% (Table 3).

DISCUSSION

The observation of a high frequency of *C. fetus* subsp. *jejuni* in the intestinal flora of healthy young cattle, sheep, and swine led to the suggestion that they are part of the normal fecal flora of immature animals (11). This may be the case with unweaned calves, and the general absence of *Campylobacter* strains in the lambs we examined is not contradictory since these animals would be about 4 months old at slaughter, with the digestive characteristics of adult animals. However, the relatively high frequency of *Campylobacter* strains in two groups of healthy adult sheep shows that healthy older animals can also carry the organisms. It is possible that *C. fetus* subsp. *jejuni* is present in animals of all ages in some flocks and herds and that their unbiquitous presence in groups of unweaned calves is due to the well-known susceptability of these animals to cross infection by enteric organisms during transport and holding before slaughter.

When animals carrying *C. fetus* subsp. *jejuni* are slaughtered, some carcasses and equipment will be contaminated by the organism. Recovery of the organism by swabbing of the carcasses is obviously inefficient, so unless destructive techniques are used, the examination of carcasses for the presence of *C. fetus* subsp. *jejuni* would seem to require sampling of extensive areas of the flank since this region is usually relatively heavily contaminated by fecal organisms. However, provided that the inefficiency of recovery is taken into account, it does seem possible to estimate the magnitude of *Campylobacter* contamination of carcasses by the swabbing techniques used. The same technique was more sensitive when applied to work surfaces, and the difference in efficiencies of recovery must be borne in mind when the results from equipment and carcass surfaces are compared. Despite these technical limitations it can be concluded that initial *Campylobacter* contamination of both carcass and equipment surfaces will generally be of the order of 1 to 10 organisms per cm^2, that the organism does not persist in the work environment, and that the numbers on carcasses probably decline during chilling. The low level of initial contamination is not surprising since *C. fetus* subsp. *jejuni* is a very minor component of the fecal flora.

Whether this low level of contamination is of any significance for human health will depend upon the dose of cells necessary to initiate *Campylobacter* enteritis and the circumstances under which the organism could grow. Attempts to induce the disease in animals by giving large oral inocula have met with only variable success (2, 6, 14), which suggests that large numbers of cells may be required for infection. However, in a recent case of human self-infection, a dose of 500 cells sufficed to establish enteritis (7). Such low doses could be ingested if raw meat were consumed, but any cooking would reduce the initial numbers substantially. Since *C. fetus* subsp. *jejuni* cannot grow at 30°C or below, it is extremely unlikely that the numbers on raw meat will increase, but there is the possibility that organisms may be transferred from raw meats to warm, prepared dishes in which growth might occur. However, there is no data on the behavior of *C. fetus* subsp. *jejuni* in food systems. Until these are available and the magnitude of the infectious dose is firmly established, it would be premature to try to assess the significance for human *Campylobacter* enteritis

TABLE 3. Recovery of *C. fetus* subsp. *jejuni* from inoculated glass and meat surfaces by swabbing 100 cm^2 and streaking directly onto selective agar[a]

Surface	Inoculum (no./100 cm^2)	No. recovered	Average recovery (%)
Glass	16	5, 2	21.9
	140	10, 17	9.6
	1,500	120, 96	7.2
	15,000	1,500, 1,500	10.0
Meat	16	0, 1	3.1
	140	2, 3	1.7
	1,500	20, 22	1.4
	15,000	152, 154	1.0

[a] All tests were duplicated.

of the contamination of red meats by *C. fetus* subsp. *jejuni*.

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